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Types of neurons of the claustrum in the rabbit — Nissl, Klüver-Barrera and Golgi studies

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The studies were carried out on the claustrum of 8 adult rabbits. Four types of neurons were distinguished: 1. Multipolar neurons, which have dendritic trunks either with conus (multipolar polygonal perikarya) or without conus (multipolar rounded perikarya). Both subdivisions of the multipolar neurons have 3–6 dendritic trunks. Only some branches of these trunks have spines. An axon emerges mainly from the cell body, rarely from the initial part of the dendritic trunk. 2. Bipolar neurons with fusiform or rounded perikarya; they have two dendrites covered with spines. An axon originates directly from the cell body or from one of the dendritic trunks. 3. Triangular neurons, which have three dendritic branches with spines. An axon emerges directly from the soma, often near the primary dendritic trunk. 4. Pear-shaped neurons with one or two dendritic trunks arise from one pole of the cell body and with an axon that originates from the opposite side of the perikaryon. The dendrites are covered with spines.

key words: claustrum, types of neurons, rabbit

INTRODUCTION

The claustrum is a subcortical structure. Two main parts can be distinguished in that neuronal centre in all mammals: the dorsal (insular claustrum) and the ventral (called prepiriform claustrum or endopiriform nucleus) [6,7,22]. The transition between them occurs at the bottom of the rhinal sulcus. The insular part is located under the insular cortex, whereas the ventral one is extended under the prepiriform cortex.

Research has shown the broad range of corticoclaustral and claustrocortical connections [10,11,16,17, 26,27]. The data concerning the function of the claustrum are not still complete. This structure is considered to integrate and analyse information which comes from the visual, auditory, somatosensory and limbic centres [4,5,11,12,15,18].

Only a few authors have described the neuronal structure of the claustrum using the Golgi technique. Types of neurons of the claustrum were investigated in human [2], rhesus and squirrel monkey [3], bison bonasus [25], cat [15,20], rat [19] and hedgehog [6]. The aim of our studies was to give full morphological characteristics of the claustral neurons in the rabbit's telencephalon.

MATERIAL AND METHODS

The studies were carried out on 8 telencephalons of adult rabbits. The preparations were made by means of the Nissl, Klüver-Barrera and Golgi methods. The brains were cut into 15 μm, 50 μm and 90 μm scraps for the Klüver-Barrera, Nissl and Golgi methods respectively. The microscopic images of the selected impregnated cells were digitally recorded by means of a camera, which was coupled with a microscope and an image processing system (VIST-Wicom, Warsaw). From 60 to 100 such digital microphotographs

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were taken at the different focus layers of the section for each neuron. The computerised reconstructions of microscopic images were made on the basis of these series. The neuropil was kept in all the pictures in order to show the real microscopic images and then was removed from each of them to clarify the picture.

RESULTS

The following types of neurons were distinguished on the basis of such criteria as the shape and size of their soma, number and arborisation of dendrites, location of an axon and distribution of tigroidal substance as well as the presence of dendritic spines.

1. Multipolar neurons (Fig. 1). They are the most numerous nerve cells in the rabbit claustrum. In this type multipolar polygonal (25–35 μm) and multipolar rounded perikarya (19–23 μm) were observed. Both subdivisions of multipolar neurons have 3–6 thick primary dendrites. The multipolar rounded perikarya have dendritic trunks without conus, whereas the dendritic trunks of the multipolar polygonal neurons arise conically. The primary dendrites bifurcate dichotomically at the distance of 10–25 μm from the cell body. The secondary dendrites and their branches have a wavy course; they are covered with bulbous spines. Most of the spines have thread-like stems, although smooth dendrites without any spines or protrusions were also sporadically observed. The dendrites spread out in all directions, making the dendritic field round or oval in shape. An axon without collaterals emerges from the perikaryon close to one of the dendritic trunks, rarely from the initial part of them. It was observed at the distance of 90 μm. Multipolar neurons have thick and medium-size granules of tigroidal matter, which surrounds an oval nucleus with a dark nucleolus. The tigroidal substance mostly penetrates into the initial portions of the primary dendritic trunks. The multipolar neurons were found in both parts of the claustrum. However, in the dorsal part the rounded perikarya were more often observed than in the ventral one. Within the claustrum there is a preponderance of the polygonal perikarya.

2. Bipolar neurons (Fig. 2). In this type with regard to the shape of perikaryon two subdivisions of cells can be distinguished: fusiform and rounded. The fusiform neurons measure 24–30 μm, whereas the rounded neurons 15–25 μm. Two primary dendrites emanate in the opposite directions from the poles of the cell body and bifurcate at the distance

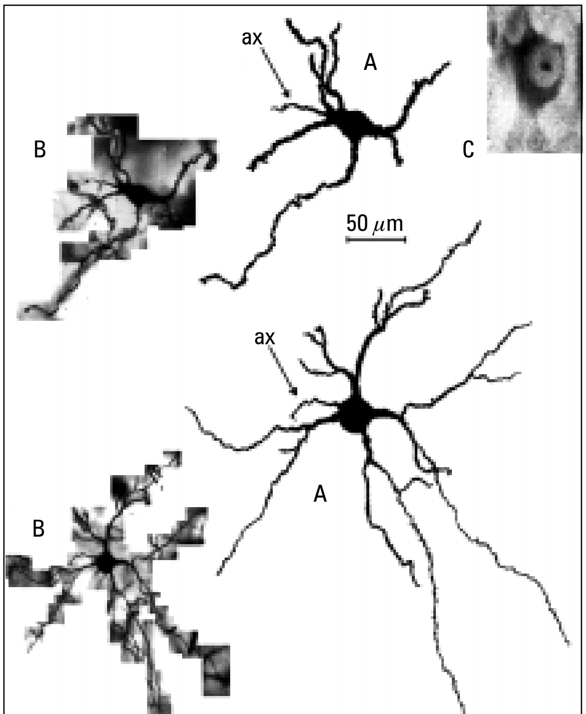

Figure 1. Multipolar neurones. A) clarified Golgi impregnation; B) non--clarified Golgi impregnation; C) Klüver-Barrera method; ax — axon.

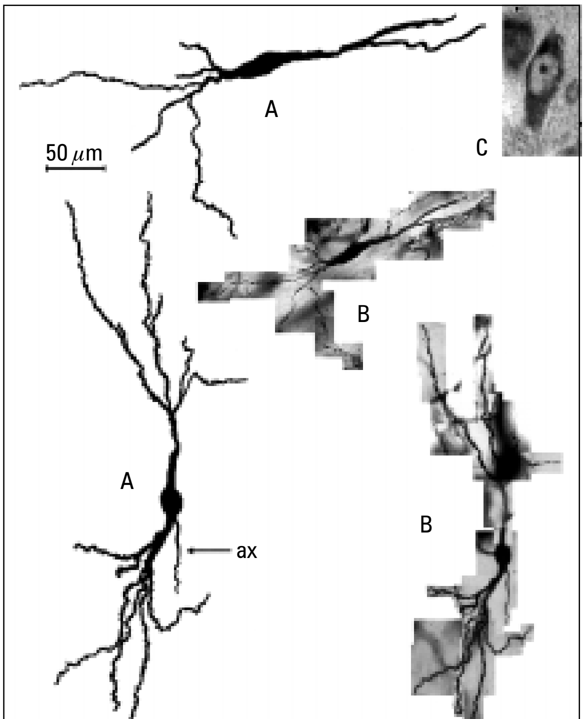

Figure 2. Bipolar neurones. A) clarified Golgi impregnation; B) non--clarified Golgi impregnatarion; C) Klüver-Barrera method; ax — axon.

of 10–40 μm. The dendrites beyond their proximal part are covered with bulbous spines. An axon originates from the thick conical elongation either from the cell body or one of the dendritic trunks. Apart from its proximal segment the axon has a relatively uniform calibre. It was observed at the distance of about 70 μm. The perikarya have spherical nuclei with intensively-stained nucleolus. A lot of thick-granular tigroidal matter penetrates into the initial portion of the dendritic trunks. In the rabbit claustrum the bipolar neurons are mostly situated on the peripheral parts of the nucleus.

3. Triangular neurons (Fig. 3). The perikarya of these cells measure from 22 to 30 μm. They have 3 thick primary dendritic trunks, which arise conically from the cell body and bifurcate dichotomically at the distance of 10–25 μm. They mostly have a smooth surface, though knob-like appendices are sporadically observed on them. The secondary dendrites and their branches have a wavy route. They are covered with big and numerous knob-like appendices. The dendritic field has an irregular shape. An axon emerges directly from the soma, often near one of the primary dendritic trunks. The cell body has a round, clearly separated nucleus with a dark-stained nucleolus. The cells contain a lot of thick and medium-size granules of tigroidal matter, which does not penetrate into the dendritic trunks. The number of the triangular neurons is similar to the number of the bipolar nerve cells. These neurons were mostly observed in the dorsal claustrum, whereas in the ventral part of the claustrum they were only occasionally found.

4. Pear-shaped neurons (Fig. 4). They have perikarya from 17 to 25 μm. One or two thick dendritic trunks emanate from one pole of the cell body. They divide dichotomically into thin secondary dendrites after 20–80 μm of their route. Most of them bifurcate once again at a different distance from the perikaryon. The secondary dendrites have a wavy route and their branches are covered with spines. Most of these spines are similar to bulbous knobs, but they are placed on thread-like stems. The dendritic field is fan-shaped. A thin axon originates from an axon-hillock, at the opposite side of the cell body. The dendrites of neurons, which have two dendritic trunks run mainly in ventro-lateral directions, but the axon usually directs dorso-medially. The course of the dendrites and the axons of the cells, which have only one dendritic trunk, is various. The soma contains the oval, indistinct nucleus. The nucleolus is intensively-stained. A lot of thick-granular tigroidal

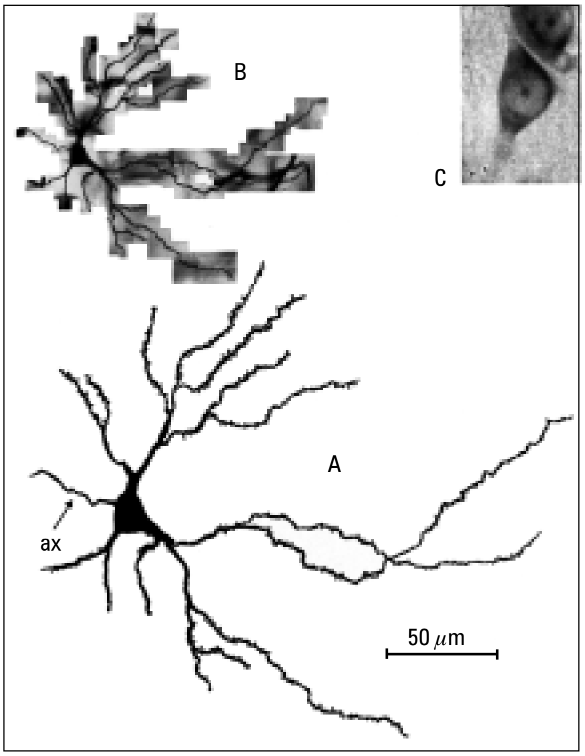

Figure 3. Triangular neurones. A) clarified Golgi impregnation; B) non--clarified Golgi impregnation; C) Klüver-Barrera method; ax — axon.

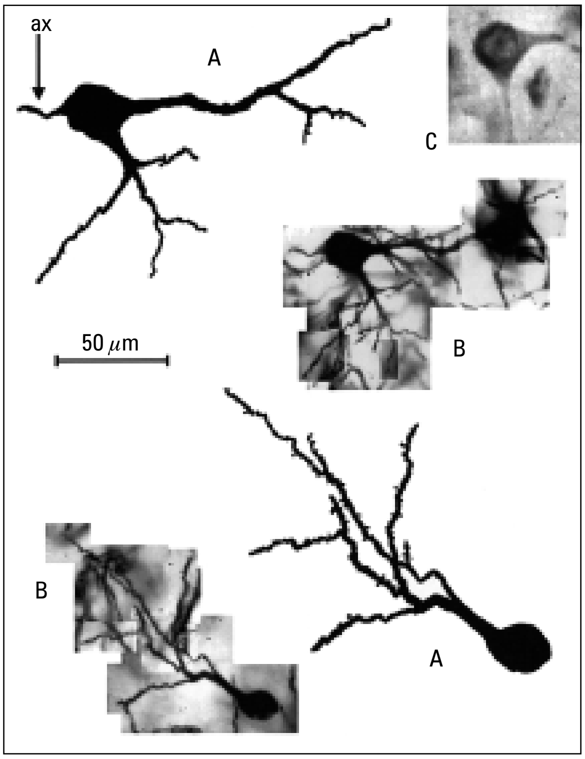

Figure 4. Pear-shaped neurones. A) clarified Golgi impregnation; B) non--clarified Golgi impregnation; C) Klüver-Barrera method; ax — axon.

substance penetrates deeply into the initial segments of the primary dendritic trunks. These neurons were the least numerous in our material. They were observed only in the dorsal part of the claustrum.

DISCUSSION

The analysis of our material shows the presence of four main types of neurons in the rabbit claustrum: multipolar, fusiform, triangular and pear-shaped. Almost all dendritic branches of these types of neurons had spines; however, among multipolar neurons smooth dendrites without any spines or protrusions were sporadically observed.

Białowąs and Chadzypanagiotis [1] using Klüver-Barrera, Nissl and Woelcke techniques distinguished multipolar, fusiform, oval neurons in the dorsal part and oval, elongated and pyramidal cells in the ventral part of the rabbit claustrum. The oval cells are similar to the rounded multipolar and the rounded bipolar neurons, found in our material. The pyramidal cells [1] seem to correspond to the triangular neurons, described in the present studies, though they differ in their body sizes. In contrast to our results Białowąs and Chadzypanagiotis [1] did not observe the pear-shaped neurons.

Dinopoulos et al. in the hedgehog [6] and Równiak et al. in the bison bonasus claustrum [25] observed two main types of nerve cells: spiny and aspiny neurons. Among the spiny neurons polygonal, triangular and fusiform cells were found, whereas among the aspiny neurons multipolar, fusiform, ovoid or round cells were described. The fusiform cells with aspiny dendrites were not distinguished in our study. The spiny multipolar neurons were the predominant cells of the hedgehog and bison bonasus claustrum, which corresponds with our results.

In the rat claustrum 5 types of cells were distinguished: MS I, MS II, MS III, MA, SA [19]. The first three types were described as spiny neurons with oval, round and triangular cell bodies. The remaining two types (MA, SA) were classified as spineless neurons with oval, round and spindle-shaped bodies. Comparing our results with these observations, we drew the conclusion that almost all types of neurons which were distinguished in the rat are very similar to the rabbit's nerve cells. However, the triangular cells observed by Mamos [19] had one main, thick dendrite, whereas our observation showed the presence of three thick dendrites. Moreover, the pear-shaped neurons were not found in the rat [19]. In the claustrum of the cat [20], where the presence or absence of spines and the number of main dendrites was the criterion of the division, five types of neurons were distinguished. Three types of spiny cells: S I — triangular and oval with one main dendrite, S II — spindle-shaped with two main dendrites, S III — multipolar, oval, round and triangular without main dendrites. Two kinds of aspiny cells: A I — cells without main dendrites, whose shapes were similar to the neurons of the previous group, A II — oval or round without main dendrites. The aspiny neurons were observed only in the posterior areas of the claustrum, mainly in its visual region [20]. All these types of neurons seem to be similar to the rabbit cells, although the aspiny neurons in our material are not so numerous. The cat visual claustrum [15] consists of spiny and aspiny neurons, and it shows a definitely lower number of the latter cells. The large spiny neurons described by Le Vay and Sherk [15] probably correspond to the multipolar rabbit cells.

Moryś et al. [21] distinguished four parts of the human claustrum: dorsal, orbital, temporal and paraamygdalar. Each of them has connections with different cortical regions. The cytoarchitectonics study demonstrated multipolar, pyramidal-like, triangular, oval and fusiform neurons. The authors [21] noticed the presence of larger cells in the dorsal and orbital parts, and the highest neuronal density in the paraamygdalar part. Data obtained from the Golgi study, revealed five types of neurons in the human claustrum [2]. Type I represents spiny nerve cells with different shapes of their perikarya. The remaining four types represent aspiny cells, varying in their size and amount of lipofuscin granules. Brand [3] in the primate claustrum described three types of nerve cells. Type I — large, spiny neurons, mainly pyramidal and fusiform, which were also observed in our material. Type II — rounded aspiny neurons. Type III — small, pear-shaped cells. The second and the third type presumably correspond with the rabbit's multipolar and pear-shaped cells, respectively.

The immunocytochemical studies in cat [23] and rabbit [9] showed the presence of GABAergic neurons in the claustrum. According to Druga et al. [8] the cell bodies of the majority of the claustral parvalbumin- or calbindin-immunoreactive neurons had oval or round shape. Their immunopositive dendritic branches had no spines.

Reynhout and Baizer [24] noticed that neurons with parvalbumin-ir were large, multipolar cells. Bipolar neurons with elongated cell bodies and beaded dendrites were immunoreactive for calretinin. Among the cells immunoreactive for calbindin, the authors [24] distinguished multipolar, bipolar, round or oval cell bodies. In spite of the absence of dendritic spines in the immunostained sections [24], these results resemble ours, which were based on the Golgi material.

Generally, there are no major morphological differences between claustral neurons in the various species of mammals, but most of the authors have not observed the pear-shaped cells. The neurons with dendritic spines are probably projection neurons [6,20,25], whereas aspiny neurons have mainly been classified as interneurons [2,3,15]. Le Vay and Sherk [15] and Kubasik-Juraniec et al. [14] observed that the majority of corticoclaustral axon terminals made synaptic contact with dendritic spines.

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